In this series, the "durum" group comprises two or three varieties that might better be listed as *T. pyramidale* Perc., a species not everywhere recognized as valid.

Of the varieties, the durum known as "Gaza" was used most extensively, crosses being made in most of the years since 1929. On three occasions there was 100% seed-setting, although in one of the very dry seasons it fell as low as 30%. No significant differences were found between varieties in regard to their compatibility with "Khapli".

Fertility of the F1 was general. Dominance of resistance was shown when these plants were tested with the same races of *P. graminis tritici* as were used with the vulgare F1s. A considerable amount of work was done on later generations, but this will be reported separately.

In addition to the 14-chromosome wheats listed, certain other species were used in preliminary tests, and gave the following results.

TABLE 2.

*Seed-setting in certain Species Crosses with "Khapli" Emmer.*

| Species. | Grains Set. | Pollinations. |
|---|---|---|
| *T. Timopheevi* .. .. .. | 21 | 24 |
| *T. Vavilovi* .. .. .. .. | 15 | 24 |
| *T. Timococcum* .. .. .. | 3 | 38 |
| *T. Sovieticum* .. .. .. .. | 1 | 34 |

Complete sterility was shown. The Timopheevi F1s gave normal vegetative growth and intermediacy of morphological characters. Leaf rust tests showed dominance of the resistance carried by the Timopheevi parent: with stem rust, to which both parents are resistant, complete resistance was shown.

Crosses were attempted with several rye varieties and with *Aegilops squarrosa*, but in no case was any grain set.

## BACK-CROSSES.

Since 1924 more than 100 back-crosses have been made. Many have involved F1s from the "Steinwedel" derivatives crossed with "Khapli" and with the vulgare parent. In other cases this F1 has been crossed with other vulgares having greater agronomic value, and in yet others, the F1 from the 14-chromosome parent crossed with "Khapli" has been back-crossed with a selected vulgare parent.

Again, material of cytogenetical value has been obtained and at the same time evidence got that the "Khapli" resistance can be transferred to vulgare wheats.

It was clearly realized from the outset that detailed cytogenetical studies were called for, and much material has been saved for this purpose. Up to the present all efforts to have these investigations made have failed. The problem is now clearly delimited, and steps should be taken to have this cytogenetical work carried out.

## ABERRATIONS IN "KHAPLI".

In the many sowings of "Khapli" made for control purposes, three albinotic plants have shown up: all died in the early stages of development.

Two striped seedlings have also been found. Both retained the striping of the leaves and came to maturity. In each case, grain gave rise to normal green seedlings, and from these at maturity bulk grain was saved which in the next generation again produced normal green seedlings.

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*References.*

HYNES, H. J., 1926.—Studies on the reaction to stem rust in a cross between Federation wheat and Khapli emmer, with notes on the fertility of the hybrid types. *Phytopathology*, 16: 809-827.

McMILLAN, J. R. A., 1933.—Varieties of wheat in Australia. C.S.I.R. Bulletin 72: pp. 29.

WATERHOUSE, W. L., 1930.—Australian Rust Studies. III. Initial results of breeding for rust resistance. PROC. LINN. SOC. N.S.W., 55: 596-636.

———, 1933.—On the production of fertile hybrids from crosses between vulgare and Khapli emmer wheats. PROC. LINN. SOC. N.S.W., 58: 99-104, 2 plates.

———, 1939.—A note on crossing wheat and rye. *Aust. Jour. Sci.*, 2: 63.

# AUSTRALIAN CLOVER RUSTS.

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*Synopsis.*

The morphology, life histories and host ranges of rusts occurring on *Trifolium* spp. in Australia, have been studied. Biometrical analyses of measurements of spore dimensions have been made. The occurrence of physiologic specialization in the rust of subterranean clover is recorded, and its importance discussed. A classification of the rusts is attempted from the results obtained.

The influence of temperature and humidity on the longevity of uredospores of subterranean clover rust has been determined.

The results are given of an experiment designed to demonstrate the effect of strains of *Rhizobium* on clover rust development.

## I. Comparative Studies.

### *Introduction.*

For many years, the rusts of *Trifolium* spp. have been studied by workers in Europe and North America. Three principal clover rust species have been differentiated on the basis of life history studies, and within one of these, three distinct subspecies differing in spore morphology and host specialization are known. The species are designated by Arthur (1934):

1. *Uromyces flectens* Lagerh., a microcyclic form on *T. repens* L.;
2. *U. nerviphilus* (Grognot) Hotson, a demicyclic form on *T. repens*;
3. *U. trifolii* (Hedw. f.) Lév., an autoecious eu-form having the following three subspecies:
   (*a*) *U. t. trifolii-repentis* (Liro) Arthur on *T. repens*;
   (*b*) *U. t. hybridi* (Davis) Arthur on *T. hybridum* L.;
   (*c*) *U. t. fallens* (Desm.) Arthur on *T. pratense* L. and *T. medium* L.

Similar studies have not been made in Australia, and since some of the predominant host species differ from those of Europe and North America, a detailed comparison of their rusts is warranted. Physiologic specialization has not been demonstrated within any clover rust subspecies. Statistical data on spore dimensions are not available.

### *Life Histories and General Morphology.*

*Literature Review*

A detailed description of the macrocyclic species *U. trifolii* is given by Arthur (1934). The differentiation of the three subspecies is set out in Table 1. Other workers, including Liro (1906), Sydow (1909), Kern (1911) and Davis (1924), have considered these rusts as distinct species, but the production of the aecidial stages of *U. t. hybridi* (Davis, 1917) and of *U. t. fallens* (Kobel, 1920) clearly established the relationship of these rusts to the macrocyclic species *U. trifolii*. For this reason, the simpler species concept adopted by Arthur is used in this paper.

The short-cycle forms on *T. repens*, viz., *U. nerviphilus* and *U. flectens*, differ little from *U. t. trifolii-repentis* in spore morphology; however the former lacks the uredospore generation, and the latter lacks spermogonia, aecidia, and uredosori. The teleutosori of the short-cycle forms are large, elongated, and mainly confined to the veins and petioles, whereas those of the macrocyclic form are small, rounded and restricted to the leaf blades.

*Clover Rusts Present in Australia.*

During the period from March, 1951, to January, 1952, five species of *Trifolium* were found rusted under field conditions, viz., *T. subterraneum* L., *T. fragiferum* L.,

*T. glomeratum* L., *T. repens* and *T. pratense*. Field, glasshouse and microscopic comparisons of the rusts showed that they differ considerably in their life cycles and general morphology. A description of each rust is given below. Brackets placed about a subspecies designation indicate that the complete classification of the rust is still in doubt.

(*a*) *U. trifolii trifolii-repentis.*

The rust most commonly found on *T. repens* produces uredospore, teleutospore, spermogonial and aecidial stages. The transient uredosori, typically small, rounded, and red-brown in colour, rapidly give place to black teleutosori. The teleutospores germinate at maturity, giving rise to the spermogonial and aecidial stages. Aecidiospores play the primary rôle in the dissemination of the organism.

The uredospores are finely echinulate, light cinnamon-brown, 19–27 × 18–21 $\mu$,* with 2–4 equatorial germpores. The spore wall is 1–1·5$\mu$ thick. The single-celled teleutospores are smooth, cinnamon-brown, 20–30 × 16–23 $\mu$, with a single terminal pore. The spore wall is 1–2$\mu$ thick.

This rust has been recorded in Australia by McAlpine (1906) under the name *U. trifolii* (Alb. & Schw.) Winter.

TABLE 1.

*Differentiation of Subspecies of* U. trifolii.

| Subspecies. | Principal Host Species. | Occurrence of Aecidia. | Uredospore Germpores. | |
|---|---|---|---|---|
| | | | Number. | Distribution. |
| *U. t. trifolii-repentis* .. | *T. repens.* | Common. | 2–4 | Equatorial. |
| *U. t. hybridi* .. .. | *T. hybridum.* | Rare. | 2–4 | Equatorial. |
| *U. t. fallens* .. .. | *T. pratense.* | Rare. | 4–7 | Scattered. |

(*b*) *U. trifolii* (*glomerati*).

The rust on *T. glomeratum* resembles *U. t. trifolii-repentis* in its life history and morphology. The uredosori, teleutosori, spermogonia and aecidia all occur frequently throughout the growing season, often on the same plant. The uredosori, as in *U. t. trifolii-repentis*, do not persist.

The uredospores are finely echinulate, light cinnamon-brown, 19–27 × 15–22 $\mu$, with 2–4 equatorial germpores. The spore wall is 1–1·5$\mu$ thick. The single-celled teleutospores are smooth, cinnamon-brown, 20–35 × 14–21 $\mu$ (somewhat longer and narrower than in *U. t. trifolii-repentis*), with a single terminal pore. The spore wall is 1–2$\mu$ thick.

(*c*) *U. trifolii fallens.*

*T. pratense* is parasitized by a rust which predominates in the uredospore stage. The uredosori are typically rounded, dark-brown, and medium in size on the leaf blades, and larger and elongated on the stems. Teleutospores are produced only under unfavourable conditions, usually towards the end of the growing season. Attempts to germinate teleutospores immediately have failed. However, material exposed to winter conditions at Glen Innes, N.S.W., showed a small proportion of viable spores. Neither the spermogonial nor the aecidial stage has been found under natural conditions.

The uredospores are finely echinulate, cinnamon-brown, 21–29 × 20–24 $\mu$, with 4–7 scattered germpores. The spore wall is 1–3$\mu$ thick. The single-celled teleutospores are smooth, cinnamon-brown, 20–29 × 15–21 $\mu$, with a single terminal pore. The spore wall is 1–2$\mu$ thick.

The occurrence of the aecidial stage on *T. pratense* has not yet been demonstrated in Australia, but the rust corresponds in all other respects to *U. t. fallens*.

* Further details of spore dimensions are given below.

(*d*) *U. trifolii subterranei.*

The rust occurring on *T. subterraneum* is similar to *U. t. fallens.* It persists almost exclusively in the uredospore stage, the rounded, dark-brown uredosori occurring on the leaflets and petioles, and on the cotyledons of young seedlings. Teleutospores are rarely produced, having been detected in only one of 46 field collections. This collection was made from a plant which had oversummered in a favourable position at Black Mountain, A.C.T. Germination tests with the teleutospores were unsuccessful, but a portion of the material exposed to winter conditions at Glen Innes showed some viable teleutospores. Inoculations of young shoots of susceptible plants in the glasshouse gave negative results. Neither the spermogonial nor the aecidial stage of the rust has been observed under natural conditions. However, the rust collection from Black Mountain contained viable uredospores, which presumably carry the rust from season to season.

The uredospores are finely echinulate, cinnamon-brown, 19–27 × 16–23 $\mu$, with 2–4 equatorial germpores. The spore wall is 1–3$\mu$ thick. The single-celled teleutospores are smooth, cinnamon-brown, 20–30 × 16–22 $\mu$, each with a single terminal pore. The spore wall is 1–2$\mu$ thick.

The presence of the aecidial generation on *T. subterraneum* has not yet been recorded, but other characteristics of the rust provide strong presumptive evidence for its inclusion in the species *U. trifolii* (Radel, 1935; Loftus Hills, 1942). Of the three subspecies, *U. t. hybridi* is most like the Australian rust, having uredospores with 2–4 equatorial germpores, and a life history in which the aecidial stage plays an unimportant part.

(*e*) *U. flectens* (*repentis*).

A rust has been found on *T. repens* under Australian conditions which is distinct from *U. t. trifolii-repentis*, being restricted to the teleutospore generation. The sori are very large, black in colour, and confined mainly to the petioles and to the midribs of the leaflets. The teleutospores germinate at maturity, the resulting sporidia causing further infection of the host. Four successive generations of teleutosori have been induced during glasshouse testing.

The single-celled teleutospores are smooth, cinnamon-brown, 18–29 × 15–23 $\mu$, each with a single terminal pore. The spore wall is 1–2$\mu$ thick. The spores germinate to give hyaline, 3–4 septate promycelia, 6–9$\mu$ in breadth, with sterigmata 4–8 × 1·5–3 $\mu$; the sporidia are smooth, hyaline, globular to kidney-shaped, and 10–18 × 5–8 $\mu$.

(*f*) *U. flectens* (*fragiferi*).

There is an Australian rust on *T. fragiferum* which resembles *U. f.* (*repentis*) very closely. Successive generations of teleutosori of the same general type have been produced under glasshouse conditions without the appearance of other spore stages. Details of spore morphology correspond to those recorded for *U. f.* (*repentis*).

In Table 2 a summary is given of the differences between the rusts parasitizing different species of *Trifolium* in Australia.

*Distribution of the Rusts.*

A total of 164 rust samples has been examined between March, 1951, and January, 1952. Of these, 140 were collected in New South Wales, 10 in Victoria, 3 in South Australia, 1 in Queensland, 1 in Tasmania, 6 in Western Australia, and 3 in the Australian Capital Territory. The localities from which the samples of each rust came are given below:

*U. t. trifolii-repentis*: Springbrook, Qld., Glen Innes, Murwillumbah, Byron Bay, Lismore, Grafton, Oxley Island, Taree, Maitland, Lawson, Sydney, Nowra and Bega, N.S.W.

*U. t.* (*glomerati*): Gosford and Castle Hill, N.S.W.

*U. t. fallens*: Glen Innes, Gosford, Richmond, Castle Hill and Bega, N.S.W.

*U. t. subterranei*: See Table 8.

*U. f.* (*repentis*): Leeton, Sydney, Castle Hill, N.S.W., Melbourne, Vic., and Canberra, A.C.T.

*U. f.* (*fragiferi*): Melbourne, Vic., and Meningie, S.A.

## *Cultural Studies.*

*Literature Review.*

The range of *Trifolium* spp. attacked by each of the three subspecies of *U. trifolii* has been studied by Liro (1906), Davis (1924), Kobel (1920), and Mains (1935). Liro and Davis attempted cross-inoculations involving the three host species, viz., *T. repens, T. hybridum* and *T. pratense*, and demonstrated that each subspecies is incapable of attacking the hosts of the other two. More extensive tests by Kobel and Mains have shown them to differ on a number of other species of *Trifolium*. The two workers' results do not in all instances agree, but the lack of correspondence is probably due to the use of different strains of the *Trifolium* spp.

The host specialization of *U. flectens* has not been determined.

TABLE 2.

*Summary of Differences in Life History and Spore Morphology shown by Australian Clover Rusts.*

| Organism. | Spore Stages Commonly Present. | Uredospore Morphology. | | | |
|---|---|---|---|---|---|
| | | Germpores. | | Spore Wall. | |
| | | Number. | Distribution. | Colour. | Thickness in Microns. |
| *U. t. trifolii-repentis* .. | Uredospore. Teleutospore. Spermogonial. Aecidial. | 2–4 | Equatorial. | Light cinnamon-brown. | 1–1·5 |
| *U. t. (glomerati)* .. .. | Uredospore. Teleutospore. Spermogonial. Aecidial. | 2–4 | Equatorial. | Light cinnamon-brown. | 1–1·5 |
| *U. t. fallens* .. .. .. | Uredospore. (Teleutospore). | 4–7 | Scattered. | Cinnamon-brown. | 1–3 |
| *U. t. subterranei* .. .. | Uredospore. (Teleutospore). | 2–4 | Equatorial. | Cinnamon-brown. | 1–3 |
| *U. f. (repentis)* .. .. | Teleutospore. | | | | |
| *U. f. (fragiferi)* .. .. | Teleutospore. | | | | |

*Materials and Methods.*

Seed of 35 species of *Trifolium* and nine species of related genera was obtained from the United States Department of Agriculture; the Pasture Research Station, Burnley Gardens, Melbourne; the Division of Plant Industry, C.S.I.R.O., Canberra; and the Department of Agriculture, N.S.W. For many of the species a number of different strains was available, and each has been studied individually.

A single rust isolate was used to represent each Australian rust type, with the exception of *U. t. subterranei*. Owing to the occurrence of physiologic specialization within this rust subspecies (to be considered in detail in a later section), two isolates were used in studies of its host range, one to represent each of the two most abundant physiologic races. The localities from which the selected isolates originated are given in Table 3.

Cultures of the rust isolates were maintained on plants of the species from which they were collected. Inoculations were made in separate glasshouses between August, 1951, and February, 1952. Seed was germinated between moist sheets of blotting paper in a 25° C. incubator, as this was found to speed up the testing work. Scarification of the seed coat with coarse emery paper improved the percentage germination. Approximately 20 seedlings were raised in sterilized soil in each 4″ pot. When six or more leaves had developed, the plants were atomized with water, and spores dusted